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A STUDY OF TESTIS AND OVARY GRAFTS ON THE HEN'S EGG AND THEIR EFFECTS ON THE EMBRYO

A DISSERTATION
SUBMITTED TO THE FACULTY
OF THE
OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

BY
TADACHIKA MINOURA

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TADACHIKA MINOURA

Zoological Laboratory, University of Chicago

ONE TEXT FIGURE AND TEN PLATES (THIRTY-SIX FIGURES)

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INTRODUCTION

The study of the free-martin by F. R. Lillie ('17 b) has thrown new light on the field of the biology of sex, demonstrating that sexual differentiation is controllable, at least to some extent, by a certain physiological factor. Lillie discovered that in the free-martin, the female of two-sexed twins in cattle, the sex-glands and sex-ducts are modified from the normal female condition to a sterile masculinized state; that in cattle twins an anastomosis of the umbilical blood-vessels exists between the two embryos, and that this anastomosis is responsible for the alteration of the female twin, the sex-hormones from the male twin being thus enabled to gain access to the female twin through the blood current and to produce a profound modification of her reproductive system. A histological study of the same material by Chapin ('17) supported Lillie's conclusion that the free-martin is a modified female. As Lillie suggested ('17 a), "the possibility exists, however, that definitely planned experiments may enable us to regulate time and dosage of hormones better than is done in this experiment of nature; the results of such experiments cannot of course be foreseen. . . . . Such experiments will be necessary for the full solution of the stated problem." I therefore, at Professor Lillie's suggestion, undertook to perform such a series of experiments for the purpose of determining whether or not sexual differentiation is experimentally controllable through the secretion of the sex-glands. Gonads were grafted on the membranes of developing chick embryos in relatively young stages; such embryos were then allowed to develop further, and in cases where the grafts grew, the urinogenital systems of the embryos were studied to determine what effects had been produced by the presence of the gonad grafts. The results of these experiments are presented in the present paper and others to appear subsequently.

It has already been abundantly proved through numerous studies on birds and mammals that the sex-glands do produce a secretion or secretions which play an important rôle in the production and maintenance of secondary sexual characters. This literature is so familiar to every one that it will not be reviewed here. With reference to birds, it may merely be said that Goodale ('16) and Pezard ('18) have demonstrated in the most convincing manner that certain secondary sexual characters of domestic birds are dependent on the secretions of the gonads.

Varying degrees of reversal of certain sex characters have been observed or experimentally obtained in both vertebrates and invertebrates by several investigators. Thus Steinach ('12, '13) described a reversal of several sex characters in the guinea-pig and rat produced by transplanting the gonads of the opposite sex into castrated or spayed individuals. His statements have been partially verified by Moore ('19). These experiments show that in these mammals certain sexual characters, both somatic and psychic, are controlled by the internal secretions of the gonads. Riddle ('16) found that in certain crosses between pigeons intersexual forms arose in correlation with a certain phase of the breeding cycle. Pearl and Boring ('18) investigated several hermaphroditic fowls and found that they possessed pathological gonads, which were probably responsible for the hermaphroditic condition. Bond ('14) reported an interesting case of a hermaphroditic pheasant of the Formosan variety which displayed male secondary characters on the left side of its body and female characters on the right side. The bird possessed a single gonad, the left one, and this was in part testicular, in part ovarian. Bond believed hormones secreted by the hermaphroditic gonad were responsible for the external hermaphroditism.

Among invertebrates the studies of Baltzer, Goldschmidt, and Gould are of great interest. Baltzer ('14) found that in the Gephyrean worm Bonellia, the indifferent larva becomes a male only when it attaches itself to a female; otherwise it develops into a female. A closely similar case was described by Gould ('17, '19) for Crepidula. In this protandric gasteropod, maleness develops only when small individuals come in contact with or remain within a short distance of a large female or transitional form. Gould was convinced by his experiments that the development of male characters is directly due to the effect on sexually indifferent individuals of a certain substance produced by large animals. Baltzer and Gould did not claim that the substances produced by the females in these cases correspond to the sex-hormones of vertebrates, but their function is evidently much like that of hormones. Goldschmidt ('16) has described intersexual forms in hybrid gypsy-moths and attempted to account for them by postulating a quantitative gradation in the value of the sex-determining factors. In his materials there was further a correlation between the degree of development of the secondary sexual characters and the degree of development of the gonads.

From investigations of this kind, two general conclusions may be drawn: first, that the sex-glands secrete certain substances which may conveniently be designated sex-hormones, which are related in an important way to the sexual characters; secondly, sexual differentiation is controllable, at least to some extent, by non-genetic factors.

The following experiments were carried out at the University of Chicago during the years 1917 to 1919. I am deeply indebted to Professor Lillie for his invaluable suggestions and criticisms during the course of the work and for his generosity in supplying me with numerous materials used in the experiments. I also wish to express my thanks to Dr. L. H. Hyman for revising the manuscript.

GRAFTS

1. *Material*

The common fowl was the material selected for the experiments, owing to the ease with which it can be operated on in embryonic stages. The white Wyandotte breed was employed in the majority of the experiments. The chicks from which the organs to be grafted were taken also belonged to this breed with a few exceptions in which other varieties of fowls were used.

2. *Experimental method*[1]

a. Operation. The surface of the developing egg selected for operation was cleaned with dilute sublimate solution or 70 per cent alcohol with or without iodine. A small piece of shell, generally 10 to 15 mm. square was then cut out from the sterilized surface, leaving the egg membranes intact. Then a V- or U-shaped slit was made through the shell membranes by means of the very sharp point of an insect pin, sterilized in the flame. The cut edge of the membranes was lifted up and a piece or sometimes pieces of the organ to be grafted placed on a desirable spot on the surface of the chorio-allantoic membrane by means of the pin point. It is probable that in this operation the chorio-allantoic membrane was injured to some extent in the majority of cases. It was observed that the most favorable place for inserting the grafts is at the junction of blood-vessels on the chorio-allantoic membrane. As quickly as possible after placing the graft, the slit in the egg membranes was closed, and the piece of shell which had been removed replaced and sealed with paraffin. Sometimes a piece of paper previously immersed in melted paraffin was used to close the opening with equally good results. The operated egg was then returned to the incubator for further development.

---

[1] This method was originally devised by Peebles ('08). It was later modified in several particulars by Murphy, Rous ('11), and Kiyono ('17).

Previous to such an operation it was of course necessary to prepare the organ which was to be grafted. Organs were removed from a chick and cut into small pieces about 1 mm. square. These pieces were kept moist in a Petri dish until the egg had been prepared for receiving them.

All of the instruments employed in the operation were sterilized in the autoclave.

The grafted organs were generally gonads. However, other organs, such as thymus, thyroid, spleen, liver, kidney, and others, were also grafted upon developing eggs in order to compare their effects upon the embryos with the effects produced by the gonads.

In each experiment generally ten to thirty eggs were operated upon and grafts from a gonad placed upon their chorio-allantoic membranes. A few control eggs were incubated simultaneously with each such lot. Some of these control eggs were treated in the same way as the operated eggs, a piece of paper or paraffin or shell being placed upon the membrane instead of a piece of organ; in other cases a window was made and closed without inserting anything on the membrane; and in still other cases the control eggs were untreated.

b. Examination. The operated eggs were removed from the incubator at different intervals after operation for examination. Such intervals varied from one day to time of hatching. In spite of the greatest care, in some experiments many eggs became infected and failed to develop further. Observations and measurements were made upon the grafts and embryos in the living state; they were then preserved for further examination. Formol-Zenker was usually employed as a fixing agent, Bouin's fluid in some cases. Many of the preserved embryos were photographed after dissection. I am greatly indebted to Doctor Bartelmez for his generosity in permitting me to use the dark room in the Anatomy Department for photographing the materials, and to Dr. Marion Hines and Mr. K. Toda for their kind assistance and advice in making the photographs.

3. Growth of grafts

a. External observations. When an operated egg is opened for examination, if the grafted piece is growing or alive, a white mass will be found on the chorio-allantoic membrane, to which umbilical blood-vessels are connected (figs. 1, 4). The mass is somewhat translucent and white or yellowish or more or less rosy in color. The grafts in general grew much more readily toward the inside than the outside of the membrane (figs. A, 5). In several instances a marked convergence of blood-vessels to the graft was noticed. Vascular connection is established with the grafts as early as twenty-four hours after operation.

The amount of growth which has occurred in the grafts is of course different in different cases depending upon the following factors: 1) duration of the graft; 2) age of the embryo at the time of the operation; 3) degree of vascular connection with the grafts. Under the most favorable conditions, the grafted masses attained a considerable size, 10 mm. in diameter and several millimeters in thickness. In the majority of cases, however, they varied from 3 to 7 mm. in diameter, that is, the original grafted piece had increased three to ten times in diameter. In table 1 are recorded a number of measurements of the size attained by grafted pieces of ovary, testis, and other organs.

TABLE 1

Length and width of grafts of ovary, testis, and other organs implanted on the chorio-allantoic membrane of the chick embryos

OVARY		TESTIS		OTHER ORGANS		
Number of embryo	Size	Number of embryo	Size	Number of embryo	Organ	Size.
	mm.		*mm.*			*mm.*
33–6	12.0 x 5.0	43–1	9.0 x 4.5	29–1	Liver	14.0 x 7.0
30–9	9.0 x 7.0	43–3	9.0 x 4.5	30–7	Thyroid	6.5 x 5.0
44–15	7.0 x 4.0	22–14	9.0 x 4.0	27–1	Thymus	6.0 x 4.5
29–11	7.0 x 3.5	22–13	8.0 x 7.0	29–8	Kidney	5.5
44–4	6.5 x 3.5	30–2	8.0 x 2.0	29–9	Thyroid and thymus	5.0 x 2.5
27–4	6.0 x 5.0	22–6	7.5 x 5.0			
19–2	6.0 x 5.0	22–12	7.0 x 5.0	29–4	Thymus	3.0 x 2.0
18–9	6.0 x 3.0	45–21	7.0			
19–1	5.0 x 5.0	22–9	6.0 x 5.0			
44–11	5.0 x 4.0	22–11	6.0 x 4.0			
17–1	5.0 x 3.0	8–5	5.0 x 4.0			
44–16	5.0 x 3.0	12–2	5.0 x 3.0			
44–14	4.5 x 2.5	47–4	5.0 x 2.5			
1–1	4.0 x 4.0	15–5	4.5 x 2.5			
44–3	4.0 x 3.5	45–6	4.5 x 2.0			
44–1	4.0 x 3.0	20–1	4.0 x 4.0			
7–1	4.0 x 3.0	22–14	4.0 x 4.0			
29–12	3.5 x 3.0	45–23	4.0 x 3.0			
41–7	3.5 x 2.5	31–3	4.0 x 2.5			
1–7	3.0 x 2.0	47–10	4.0 x 2.5			
6–2	3.0 x 2.0	30–8	4.0			
29–7	3.0 x 1.5	17–7	3.5 x 2.0			
29–5	3.0 x 1.5	34–5	3.0 x 3.0			
30–6	2.5 x 2.0	27–5	2.0 x 2.0			
42–6	2.5 x 1.8	45–15	2.0 x 2.0			
41–9	2.5	28–8	2.0 x 1.5			
17–5	2.0 x 2.0	45–18	2.0 x 1.0			
7–4	2.0 x 1.0	27–6	2.0 x 1.0			
2–5	1.5 x 1.0	30–4	1.5 x 1.5			

In table 2 the sizes attained by ovary and testis grafts are compared. From these tables it is evident that testis grafts grow somewhat larger than ovary grafts and that grafts of other organs undergo a greater growth than grafts from either gonad.

Kiyono ('17) grafted tissues and organs on chick and duck embryos and classfied them according to their capacities for growth into three groups, the first named having the greatest capacity: 1) connective tissue, cartilage, skin; 2) muscle, mucous membrane of the alimentary and respiratory tracts; 3) kidney, eye, liver, nervous tissue, and glands. He stated that tissues and organs having simple functions grow most readily when grafted, while those having more complex structure and functions grow less easily. This statement was verified in my experiments, since I found that ovary and testis grafts have a lower growth capacity than thymus, liver, and spleen grafts.

TABLE 2

Comparison of sizes attained by testis and ovary grafts

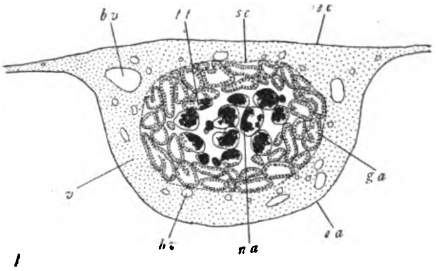
SIZE	NUMBER OF TESTIS GRAFTS	NUMBER OF OVARY GRAFTS
mm.		
Over 10	0	1
9–10	3	1
8–9	2	0
7–8	3	2
6–7	2	4
5–6	3	4
4–5	9	5
3–4	2	6
2–3	5	5
1–2	1	1

b. *Histological study.* The histological structure of sectioned grafts was studied with great interest. In general, sections of the grafts exhibited four distinct regions arranged concentrically (fig. A).

1. Chorio-allantoic membrane. The graft is encircled by the chorio-allantoic membrane. The external layer of this membrane consists of the ectoderm of the chorion; it passes over the outer surface of the graft (fig. A, *ec*). The internal layer passing beneath the graft is the entoderm of the allantois (fig. A, *ea*). Both ectodermal and entodermal membranes are very thin layers, one cell in thickness. Between the ectoderm and entoderm is the mesoderm, composed of the fused mesoderms of both chorion

and allantois. The graft lies in this mesoderm. The chorio-allantoic membrane appears in general nearly normal in structure, but is irregularly bounded and its cells somewhat more compact than is normally the case.

2. Vascular area. Within the membranes just described and encircling the graft proper is a vascular layer. This consists originally of a stroma of mesoderm cells richly infiltrated with small blood-vessels, but with the growth of the graft the stroma is greatly increased in quantity. It is composed of spindle-shaped connective-tissue cells and of fibers; it is somewhat more loose in structure than the normal stroma. Blood-vessels are distributed abundantly through the stroma and show a tendency to aggregate toward the center of the graft. Intermixed with the stroma structure one finds numerous leucocytes, singly or in groups. Some of these are hemoblasts or lymphoblasts; others are granuloblasts, granulocytes, or polymorphonuclear leucocytes. Many of them contain granules which stain with hemotoxylin and eosin; these are polymorphonuclear. Mitotic figures are not uncommon. Of course this abnormal accumulation of leucocytes around the graft is a response to the presence of foreign tissue.



Text fig. A Diagram of the structure of a growing graft (testis). *ec*, ectoderm of chorion; *ea*, entoderm of allantois; *bv*, blood-vessel; *va*, vascular area; *ga*, growing area; *sc*, connective-tissue sheath; *na*, necrotic area; *tt*, transitional tubules, regenerating at one end and necrotic at the other.

3. Growing area of the graft. This is naturally the most important region of the graft—the area occupied by the growing tissues of the graft. In the case of testis graft one finds in this

area numerous growing seminiferous tubules, having normal cell arrangement and appearance. Mitotic divisions in the cells of the seminiferous tubules are readily and frequently observable in these testis grafts and their presence demonstrates beyond doubt that an active growth of the seminiferous tubules had occurred (fig. 6). Proliferation of endothelial cells of the stroma between the tubules is also often observable. In some cases around this new growth, that is, between it and the surrounding mesenchymatous stroma, a sheath of connective tissue, resembling the normal albuginea, had been formed (fig. A, *sc*). Study of serial sections showed that the seminiferous tubules had already assumed the convoluted form.

In the case of ovary grafts, the growing area consists of numerous follicles, each composed of a central ovocyte surrounded by a single layer of cuboidal granulosa cells, very much as in the normal ovary (figs. 2, 3).

4. Necrotic area. This consists mainly of a mass of necrotic tissue, but there is no distinct boundary between this and the preceding area. The size of the necrotic center varies in different grafts. As the new growth is always due to a regeneration from the original grafted piece of tissue, one finds, as a rule a gradual transition from the center of the necrotic area to its periphery, from necrotic to living tissue (fig. A, *tt*). Thus in testis grafts one end of a tubule will consist of living or regenerating cells, while the other end terminates in a degenerating mass. The necrotic changes involved in many cases pycnosis or karyorrhexis, in other cases, karyolysis. The degree of necrosis developed in the center of the graft is dependent of course upon the rapidity with which a vascular supply to the graft develops. The slower the establishment of vascular and nutritive connections between the graft and the embryo, the more extensive are the necrotic changes in the graft. In some cases the central tissue mass was almost liquified and absorbed by phagocytosis, as was demonstrated by the existence of giant-cells around the tissue debris. Tke position and number of necrotic centers varies in different grafts, and on this basis the grafts may be classified as: monocentric, in which case the necrotic area may be either centrally located or acentric, dicentric, and polycentric.

c. Comparison of growth of testis grafts and ovary grafts. It was generally found that testis tissue grew faster and more readily than ovary tissue. Although the ovary grafts as a whole exhibited considerable growth, the amount of new growth, that is of follicular ovarian tissue, was rather small as a rule. In the case of testis grafts, on the contrary, a well-marked new growth of testis tissue was often found, in spite of the smaller size of the grafts as a whole. In other words, ovary tissue shows a greater tendency toward necrosis and less tendency toward growth and differentiation than testis tissue. We cannot at present give any explanation for this difference. It may be suggested, however, that under the same physiological conditions ovary tissue requires a more rapid or more abundant nutritive supply than testis tissue, or that ovary tissue is less resistant to a foreign environment than testis tissue, or that both factors are are responsible.

TABLE 3

Showing age of the embryos at the time of grafting and number of embryos of each age which were affected by the presence of the graft

AGE OF EMBRYOS AT TIME OF OPERATION	NUMBER OF EXPERIMENT	NUMBER OF AFFECTED EMBRYOS		
		Markedly affected embryos	Slightly affected embryos	Total
days				
2	35	0	0	0
4	9, 25, 29, 36	0	1	1
5	8, 35	1	2	3
6	28, 42, 43	1	1	2
7	2, 3, 7, 26, 32, 45, 46	7	7	14
8	1, 18, 20, 21, 36, 41, 47	3	17	20
9	10, 27, 30, 31, 35, 40	7	3	10
10	15, 33, 44	2	12	14
11	14, 19, 22, 28, 29, 34	5	14	19
12	46	0	0	0
13	17, 18	4	0	4
16	30	0	0	0

d. Age of the embryo most suitable for grafting. The organs which were grafted were taken from birds of various ages, from nine-day-old embryos up to adult fowls. The ages of the embryos upon which the grafts were implanted are given in table 3.

As shown in table 3 the grafts produced the most effect when they were implanted on the embryos during the second week of incubation, that is, from the seventh to the thirteenth days of incubation. Especially 8-, 9-, 10-, and 11-day old embryos were found to be most fmvorable for the purpose of the experiments. The facts revealed by table 3 are not difficult to understand. In embryos younger than five days the allantoic circulation is not sufficiently well established to permit of vascular connections with the graft; grafts inplanted on such young embryos therefore die from a lack of blood supply. In the case of embryos more than two weeks old it is probable that a resistance to foreign tissues has been developed and that such embryos are thereby enabled to destroy the grafts. Murphy ('14) pointed out that a defensive mechanism against foreign tissue develops in chick embryos at about the time of hatching. He noted a sudden appearance of lymphoid cells around the graft at this time and ascribed the function of the destruction of foreign tissue to these cells, considering them to be similar in function to the cells of the spleen and of bone-marrow. My results are in general agreement with Murphy's statements except that in the case of my experiments I am unable to determine whether the resistance to foreign tissue appears suddenly or develops gradually. It is, however, certain that embryos more than two weeks old are unfavorable for the implantation of foreign tissue.

ANATOMICAL STUDY OF THE EMBRYOS

We may now proceed to a consideration of the effects of the gonad grafts on the anatomy of the chick embryos. In this paper only the gross anatomy of the affected embryos will be described, the histological findings being reserved for a subsequent paper.

1. Development of sex-glands and sex-ducts in normal embryos

Before passing to a consideration of the experimental results, it is necessary to review the normal development of the sex-glands and sex-ducts in the chick, with special reference to those points concerned in the present experiments.

a. Sex differentiation. R. Semon ('87) described the differentiation of sex in the chick in the following words:

> Die geschlechtliche Differenzierung, die sich beim Hühnchen am 5., spätestens am 6. Tage erkennen lässt, macht sich zunächst dadurch bemerklich, dass bei weiblichen Embryonen die rechte Keimdrüse in auffallender Weise im Wachstum zurückbleibt. Auch beim Männchen entwickelt sich häufig der rechte Hode langsamer und bleibt oft zeitlebens kleiner. Aber die Differenz ist hier stets so unbedeutend, dass man am 6. Tage immer ohne Mühe Hode und Eierstock an diesem Merkmal unterscheiden kann.

Later Swift ('15), on histological grounds, stated:

> When the chick embryo has reached the 156th hour of development (6½ days), the formation of cords of first proliferation ceases rather abruptly and it is about this time that the sex of the individual can be definitely determined. In the determination of sex there are three criteria on which reliance can be placed. These are the relative size of the two gonads, the germinal epithelium, and the number of primordial germ-cells in the germinal epithelium. . . . an interesting fact and one to which attention has not been previously called, is the presence of more primordial germ-cells in the germinal epithelium of the left female gonad than in the epithelium of the male gonad.

From these two quotations it is evident that the first signs of sexual differentiation, both micro- and macroscopic, appear between the fifth and seventh days of incubation. The most readily recognizable character is the difference in size between the right and left gonads in the female. Besides this size difference, a difference in shape between male and female gonads is also noticeable. In some cases the retrogression of the müllerian ducts in the male had already begun on the sixth day. We may therefore say in general that sexual differentiation appears toward the end of the first week of incubation.

b. Degeneration of the right ovary. Both ovaries develop equally until the end of the first week of incubation, that is to say, the development of the ovaries is symmetrical up to the time when sexual differentiation appears. But from this time on the development of the two ovaries is asymmetrical. The left ovary continues to increase in length and size, while the right ovary undergoes a process of degeneration and finally disappears, usually before hatching. Although the degeneration of the right ovary in birds has been known for a long time, there are, curiously enough, no detailed studies upon the matter, so that there is in the literature no information available concerning the degree of development attained by the right ovary, the time at which it begins to retrogress, or the manner and degree of its retrogression. From my work I am able to add some details to the facts already known. After the stage when the difference between the two ovaries becomes apparent, the right ovary shows some slight increase in length, usually not more than 50 per cent. This continues until about the end of the second week of incubation. From this time on, a process of degeneration is manifested in the right ovary by its decrease in length and change in position. Up to the sixth or seventh day the two ovaries lie nearly parallel to the longitudinal axis of the body. After the right ovary has begun to degenerate, its anterior end tends to incline more and more to the right side, so as to assume an oblique position with reference to the right wolffian body. The outlines of the ovary gradually become less and less distinct; this change is more marked on the medial and anterior margins of the ovary. Meantime the volume of the ovary continually diminishes so that it becomes more and more slender. In the case of several 18 to 20-day-old females, the right ovary was reduced to a flattened membranous body, although the fading outlines could still be determined. The final disappearance of the right ovary occurs in general at about the age of 18 days; individual differences, of course, exist. The data on the length of the two ovaries at various stages of development are given in table 4.

c. *Wolffian ducts.* It has generally been believed that in the female chick embryo the wolffian ducts degenerate simultaneously with the wolffian bodies before hatching, or, at least, at some later time. The recent observations of Goodale ('16) Boring and Pearl ('18), however, show that this belief is erroneous. Goodale states that "apparently the wolffian duct and body may not always degenerate in the female." Boring and Pearl dissected a number of newly hatched chicks or chicks

TABLE 4

Length of right and left ovaries at various ages

AGE OF EMBRYO	LENGTH OF LEFT OVARY	LENGTH OF RIGHT OVARY
days	*mm.*	*mm*
7	1.5	1.5
9	2.2	1.6
10	3.2	2.2
11	3.7	2.0
12	3.5	2.0
13	3.0	1.3
	3.0	1.5
	3.3	1.7
14	5.0	2.0
15	4.3	1.8
16	4.3	1.5
17	4.3	1.5
	4.3	1.5
	4.5	1.3
	4.6	1.5
	5.0	1.8
18	4.0	1.5
	4.8	0.0
	5.0	2.0
	5.0	2.0
	5.0	0.0
	5.0	0.0
	5.2	1.5
20	5.0	1.0
	5.2	0.0
	6.0	0.0

from pipped eggs, seven of which were female, and five laying hens, and found that all of them had wolffian ducts. My own observations agree with those of Boring and Pearl. I have examined hundreds of embryos, and in all females without exception, the wolffian ducts were present. The wolffian bodies are always found in a degenerating condition as the time of hatching approaches and in some cases have almost completely disappeared at this time, but the wolffian ducts persist.

It is therefore certain that the presence of the wolffian ducts cannot be used as a criterion of the male condition. Whether the wolffian ducts eventually disappear in very late adult life and whether they have some function during their existence in the female are not known at the present time.

A careful study was made to determine whether or not the wolffian ducts are equally developed during embryonic life in the two sexes and whether or not the right and left ducts in each sex are of the same dimensions. It was found that in the male the posterior portion of the wolffian ducts is generally of greater diameter than in the female. This difference is noticeable in the second week of incubation in most, if not all, cases. It was further observed that in both male and female embryos after the second week the left duct, especially near its posterior end, or near its connection with the cloaca is distinctly larger than the right duct. Sometimes the left duct of the male is nearly as large as the ureter, while the right duct is always smaller than the ureter. In the female the left duct generally runs dorsal to the müllerian duct near its posterior end and is bound to the latter by connective tissue.

These points of difference between the wolffian ducts of male and female, although slight, can nevertheless be utilized as distinguishing sexual characteristics.

d. Müllerian ducts. The fate of the müllerian ducts in embryonic life is quite characteristic in each sex. In the male these ducts on both sides do not develop very far, but gradually retrogress and always disappear before hatching. In his book "The Development of the Chick," Lillie states that "Retrogression begins posteriorly and proceeds in the direction of the head; the ostium is the last to disappear." My observations agree in general with this statement. The process of retrogression does not proceed regularly from the posterior end, but begins in one or several places in the ducts irregularly so that intact portions are left between thread-like degenerated portions. The anterior end at the level of the wolffian body remains intact longer than other regions of the ducts in most embryos. The time at which retrogression begins is more or less variable; sometimes the process has already begun in six-day old embryos while in other cases one duct, but generally not both, may still persist in twelve or thirteen-day-old embryos in a nearly intact condition. In the majority of cases the right müllerian duct retrogresses more rapidly than the left one. Both ducts completely disappear between the seventeenth day of incubation and the time of hatching.

In the female, the left müllerian duct persists and continues to develop, eventually differentiating into a functional oviduct. The process of degeneration of the right duct is, however, quite different from that in the male.

The degeneration of the right müllerian duct was investigated by Gasser ('74). Gasser states that the duct ceases to develop soon after the eighth day; that by the twelfth day it is no longer present in the region of the sex-glands; by the fifteenth day it has disappeared from the region occupied by the wolffian body, and that after this time there remain only traces of the duct along its course together with a small lumen close to the cloaca. According to my observations, however, the degeneration of the duct does not take place as regularly as described by Gasser. In the first place it is somewhat doubtful that the development of the duct ceases after the eighth day; a differentiation of the posterior end, a widening similar to that occurring in the left duct, is always noticeable subsequent to this time. In fact, this differentiation of the posterior end may continue for a considerable length of time, even though degeneration is progressing at the anterior end. Consequently, in advanced stages, after retrogression is completed, the posterior end of the right müllerian duct is still present as a short widened tube with a small lumen near the cloaca. This differentiation of the posterior end of the right müllerian duct of the female does not occur in the male, even to the slightest degree. In the second place, the degeneration was found to occur somewhat irregularly. In many cases, in embryos as young as eleven or twelve days, the right duct was already in an advanced state of degeneration. On the other hand, seventeen-day-old embryos may retain more than half of the duct intact.

e. *Range of size variation in normal testes.* Data on the normal size variation of the reproductive glands and ducts in birds are rather scanty. Almost no attention has been paid to the variation in the size of the two testes, it having apparently been assumed that the testes of the two sides develop equally.

Etzold ('91) measured the testes of the sparrow and found that the left testis is larger than the right one. Swift ('16) made the same observation in the chick embryo at the age of six days and noticed further that there are more germ cells in the left testis. Riddle ('17) studied this matter very carefully in the pigeon and found that the right testis normally weighs more than the left testis. In regard to the shape of the two testes, he states that "the left is thinner and more elongated, the right shorter and thicker." In regard to this difference in shape between the two testes, Riddle made an interesting suggestion: "This difference in form is perhaps not without interest since the only persistent gonad in the female—that of the left side—is characteristically 'thin' and 'long.' The testis that develops on this side is similarly characterized as compared with its mate of the right side." Riddle also determined the weight of the testes in the fowl, but owing to the small number of individuals investigated, the results were indefinite.

I have collected some data on the development, size, and shape of the two testes in chick embryos. Both testes develop rapidly until about the eighteenth day, after which their rate of development diminishes. They are about equal in size, or the left one may be a little larger. This inequality in the size of the two testes appears more clearly in the earlier stages of development and tends to disappear later. In the majority of cases, the difference in length of the two testes ranges between 0.1 and 0.3 mm. In normal embryos the difference does not exceed 0.5 mm. The shape of the two testes is similar to that described by Riddle for the pigeon. The left testis is more elongated than the right one; the right one is shorter and in a few cases thicker. But in general I cannot say that the right testis is the larger of the two. Owing to the nature of my experiments, I could not remove the testes for the purpose of weighing them, but I am inclined to think that in the chick the left testis is larger and weighs more than the right one. The data concerning the lengths of the right and left testes of the chick are given in table 5.

2. Development of sex-glands and sex-ducts in grafted embryos

a. General résumé of the experimental results. Nearly one thousand eggs were used in the experiments. Of these about three hundred were incubated as controls and about one hundred more were used for grafting organs other than gonads. The remaining eggs served for the implantation of pieces of ovary and testis. In all 187 embryos on which gonad grafts had been implanted were living at the time of examination, and these furnish the materials for this part of the paper.

The 187 embryos obtained in the experiments are classified in table 6. In this table and elsewhere testis graft means that a piece of testis had been grafted on the membranes of the embryo in question and ovary graft that a piece of ovary had been so grafted. Male-type embryos are those which have predominantly male anatomical characteristics. The normal male at or near the time of hatching is distinguished by the following characters: there are two testes of equal size; the testes are elongated in form and smooth in texture, and there are no müllerian ducts (figs. 7, 8). Affected embryos whose gonads and ducts approach this description are hence regarded as male-type embryos. Female-type embryos are those whose gonads and ducts resemble those of the normal female. The characteristics of the normal female at or near the time of hatching are: the right ovary is absent; the left one is larger and broader than either testis; its surface is commonly rough in texture; the left

TABLE 5

Length of right and left testes of the normal chick embryo at different ages

AGE OF EMBRYO	LENGTH OF LEFT TESTIS	LENGTH OF RIGHT TESTIS	DIFFERENCE IN FAVOR OF LEFT TESTIS
days	*mm.*	*mm.*	*mm.*
7	2.8	2.7	0.1
13	3.0	2.8	0.2
	3.0	3.0	0.0
14	3.0	3.1	−0.1
15	3.2	3.0	0.2
	3.2	3.3	−0.1
	3.7	3.5	0.2
16	3.2	3.3	−0.1
17	3.0	3.0	0.0
	3.0	3.0	0.0
	3.7	3.5	0.2
18	3.3	3.3	0.0
	3.7	3.8	−0.1
	3.8	3.5	0.3
	3.8	4.0	−0.2
	4.0	4.0	0.0
	4.7	4.5	0.2
19	4.3	4.0	0.3
20	4.0	4.0	0.0
	5.0	5.0	0.0
21	4.0	4.0	0.0

TABLE 6

Classification of all experimentally obtained embryos

	MARKEDLY AFFECTED EMBRYOS (INTERSEXES)	SLIGHTLY AFFECTED EMBRYOS		APPARENTLY NON-AFFECTED EMBRYOS		TOTAL
		Male type	Female type	Male	Female	
Testis grafts..........	16	13	20	23	32	104
Ovary grafts.........	14	15	10	17	27	83
Total..............	30	58		99		187

müllerian duct is greatly enlarged and differentiated in the direction of the adult oviduct; the terminal portion of the right müllerian duct is generally still present (fig. 9, 10). Affected embryos whose gonads and ducts are similar to this condition are regarded as female-type embryos. It is to be understood that the gonads and ducts of the affected embryos exhibited all possible gradations between apparently normal embryos and intersexual conditions, in which cases, the embryos could not be definitely assigned to either sex.

In table 6 the experimentally obtained embryos are classified into three categories: markedly affected embryos, slightly affected embryos, and those apparently non-affected. Since, however, all gradations exist between these three classes, it is to be understood that the assignment of some of the embryos into such categories is largely arbitrary.

In general it may be said that whenever the grafts exhibited a good growth, the embryos were affected, but when the grafts grew slightly or not at all the resulting embryos were nearly normal or apparently non-affected.

I shall now describe in detail the anatomical characters of a number of affected embryos, selecting those which showed distinct alteration of their urinogenital systems toward an intersexual condition. In these descriptions each embryo is designated by a double number, the first one referring to the number of the experiment, the second to the number of the particular embryo in that experiment. For instance, embryo 27–6 designates the sixth egg operated on in the twenty-seventh experiment.

b. Description of embryos affected by testis grafts.

1. Embryo no. 27–6 (fig. 11, 12). Operated May 7, 1918. One-fifth of the left testis of a one-month old chick was grafted on the membranes of an embryo nine days old. Testis 5 mm. long; grafted piece about 1 mm. square. Examined May 17th; embryo developing and graft with vascular connections, of small size, however. Age of embryo, 19 days; duration of graft, 10 days.

Result: Right gonad of affected embryo 4.1 x 1 mm.; left gonad 4.1 x 0.8 mm.; peculiar black pigmentation over half of left gonad. Wolffian ducts of male type; müllerian ducts present on both sides. The left müllerian duct is about 20 mm. long; right about 9 mm., the anterior part of the right duct having disappeared as in the female. The posterior portions of both ducts are distinctly expanded; the expanded portions of the left duct measures 7 x 1.5 mm., that of the right duct, 5 x 1.2 mm. The ostium of the left duct and the differentiation of its posterior part into uterus and shell gland are nearly the same as in the normal female. But at least two parts, the one near the posterior end of the left wolffian body and the other about 3 to 4 mm. anterior to the expanded portion, show slight signs of degeneration; that is to say, decrease in diameter of the duct. It is evident, then, that this embryo possesses müllerian ducts of the female type but gonads of the male type. It is therefore in a typical intersexual condition.

2. Embryo no. 31–3 (fig. 13, 14). Operated June 14, 1918. Both testes (left 3.8 mm. long, right 4 mm. long) of a two-weeks-old chick were grafted on the membranes of an embryo of eight and one-half days' age. Opened June 24th. The two grafts were found with vascular connections. Age of the embryo, nearly 19 days; duration of graft, 10 days.

Result: Gonads of the male type, both 3.5 mm. long. Müllerian ducts present on both sides, the left one 22 mm., the right one 20 mm., in length; posterior portions of both show signs of enlargement. The right duct gradually increases in size posteriorly and the left one shows marked posterior differentiation. A short middle portion of the left duct exhibits signs of degeneration. This embryo, like the preceding one, has gonads of the male type, but the general condition of the müllerian ducts is decidedly female-like, although not so strikingly so as in the preceding case.

Attention may be called to the fact that in these two embryos, nos. 27–6 and 31–3, which are the most highly modified ones obtained, the gonad graft was implanted on the embryo when the latter was in a relatively early stage of sex differentiation.

3. Embryo no. 47–10 (fig. 15). Operated July 19, 1919. Two pieces of testis from a week-old chick were grafted on an

eight-day embryo. Opened August 1st. Grafts growing, but small. Age of embryo, 21 days; duration of graft, 13 days.

Result: Gonads of the male type, the left one 3.5 x 1.1 mm., the right one 3 x 1 mm. They have, however, an abnormal position on the wolffian bodies; instead of being parallel to the median sagittal axis, they are inclined 50 to 60 degrees to it. Wolffian ducts small, of the female type. Both müllerian ducts present, and both with slight posterior differentiation as a uterus. This embryo has male-like gonads, but female type ducts.

4. Embryo no. 43–3 (fig. 16). Operated March 14, 1919. Piece of testis about 1 mm. square from a chick thirty-six days old implanted on an embryo of six days. Opened for examination March 29th. Graft showed good growth. Age of embryo, 21 days; duration of graft, 15 days.

Result: Gonads of the male type; left one 3.5 mm. long; right 5.7 mm.; anterior two-fifths of the left gonad pigmented. Wolffian ducts rather of the male type; müllerian ducts present on both sides and exhibiting only slight expansion of their posterior portions.

5. Embryo no. 27–5 (fig. 17). Conditions of the experiment same as under embryo no 27–6.

Result: Gonads of the male type; left, 3.7 x 1.4 mm.; right, 3 x 1.3 mm. Wolffian ducts of the male type. Anterior portions of the müllerian ducts present; posterior two-fifths of their courses degenerating on both sides. Such persistence of the anterior portions, and the shape of posterior portions (although they are retrogressing) show female rather than male characteristics, or at least indicate an original development in the female direction later inhibited.

6. Embryo no. 28–8. Operated May 18, 1918. The right testis of a chick embryo one week old was grafted on an embryo of eleven days. Opened May 27th. Age of embryo, 20 days; duration of graft, 9 days.

Result: Gonads of the male type; left 4.5 x 1.1 mm.; right, 3.5 x 1.3 mm. Wolffian ducts of the male type. The müllerian ducts have degenerated, leaving slender thread-like remnants along their original courses. As a whole this embryo is more male-like than those described already; but the size relations of the two gonads is abnormal, the left one being larger than the right.

7. Embryo no. 45–18 (fig. 20). Operated May 1, 1919. Piece of adult testis (bird six or more months old, testis 18 mm. long) grafted on a seven-day-old embryo. Opened May 13th. Age of embryo, 19 days; duration of graft, 12 days.

Result: Gonads of the male type, but the left one is very much larger than the right: size of left gonad, 4.3 x 1.2 mm.; size of right one, 3 x 1.2 mm. The wolffian ducts are not very characteristically male. A slight trace of the anterior part of the left müllerian duct present; posterior part only of the right duct present and attached to the cloaca as in the normal female.

8. Embryo no. 45–6 (fig. 19). Experimental conditions the same as in no. 45–18.

Result: Gonads of the male type, but the left one larger than the right, as in no. 45–6; left gonad, 4.2 x 1 mm.; right gonad, 3 x 1 mm. Wolffian ducts more or less of the female type; only traces of the müllerian ducts present.

9. Embryo no. 15–5 (fig. 18). Operated November 13, 1917. Piece of adult testis grafted on an embryo ten days old. Opened November 22nd. Age of embryo, 19 days; duration of graft, 9 days.

Result: Left gonad of the male type, 5 x 0.9 mm.; right gonad more or less of the female type, 2.7 x 1 mm. Wolffian ducts of the male type. Posterior portions of the müllerian ducts present on both sides, about 5 mm. in length; the left duct somewhat differentiated in the female direction.

10. Embryo no. 10–5. Operated October 3, 1917. Piece of adult testis grafted on a nine-day-old embryo. Opened October 23rd. Age of embryo, 19 days; duration of graft, 10 days.

Result: Right gonad of the male type, 3 mm. long; left gonad not typically male in form, but flattened somewhat like the female type gonad, 4.8 mm. long. Left gonad markedly larger than the right. Wolffian ducts of the male type. Condition of the müllerian ducts could not be made out, owing to poor preservation.

11. Embryo no. 45–23. Conditions of the experiment the same as in nos. 45–18 and 45–6. The graft exhibited marked growth and possessed well-established vascular connections.

Result: Gonads of the male type; left gonad, 3.3 x 0.8 mm.; right gonad, 2.8 x 0.7 mm. The left is longer and the texture of its surface different from that of the right. Wolffian ducts rather of the male type; no trace of the müllerian ducts.

12. Embryo no. 8–5. Operated August 29, 1917. Adult testis grafted on an embryo of five days. Opened September 11th. Age of embryo, 18 days; duration of the graft, 13 days.

Result: Gonads of the male type; left gonad, 3.5 mm., right gonad 2.5 mm. in length. Wolffian ducts of the male type. Anterior portion of the right müllerian duct and posterior portion of the left one present.

13. Embryo no. 22–13 (fig. 22). Operated December 19, 1917. Piece of adult testis grafted on an embryo of eleven days. Opened December 26th. Graft showed good growth, 7 x 8 mm. in dimensions. Age of embryo, 18 days; duration of graft, 7 days.

Result: Gonads of the female type in general; left gonad, 4.5 x 1.5 mm.; right gonad, 3 x 1 mm. The right gonad is markedly larger than is the case in the normal female of this age. Wolffian ducts of the male type; müllerian ducts of the female type.

14. Embryo no. 17–7 (figs. 23, 24). Operated November 19, 1917. Two pieces of testis from a week-old chick were implanted on a thirteen-day-old embryo. Examined November 26th. Age of embryo, 19 days; duration of graft, 6 days.

Result: Gonads of the female type; left gonad, 4.5 x 1.7 mm.; right gonad, 3.3 x 0.5 mm. Right gonad larger and more distinctly outlined than in normal females of the same age. Wolffian ducts more or less of the female type; müllerian ducts of the female type, but their posterior ends not differentiated to the normal degree.

15. Embryo no. 45–15. Conditions of the experiment the same as in nos. 45–18 and 45–6.

Result: Gonads of the female type, but right gonad larger than is normally the case; left gonad, 4.5 x 1.8 mm.; right gonad, 2.3 x 0.8 mm. Wolffian ducts more or less of the male type; müllerian ducts of the female type.

16. Embryo no. 22–9. Conditions of the experiment the same as in no. 22–13. The graft grew well. Age of embryo, 19 days; duration of graft, 6 days.

Result: Gonads of the female type; left gonad 6 mm. long; right gonad, 3 mm. long. Wolffian ducts of the male type; müllerian ducts of the female type.

c. *Description of embryos affected by ovary grafts.*

1. Embryo no. 27–4 (figs. 25, 26). Operated May 7, 1918. Piece of ovary about 1 mm. square from a chick one month old was grafted on a nine-day-old embryo. Examined May 17th. Graft with vascular connections, but rather small. Age of embryo, 19 days; duration of graft, 10 days.

Result: Gonads of the male type, but ducts of the female type. Right gonad, 3.2 x 1.4 mm.; left gonad, 4 x 1.2 mm. Gonads testis-like in appearance, but the left one much the larger. Both müllerian ducts abnormal; the posterior portions were distinctly differentiated as in the female; there were no signs of degeneration in the ducts such as occur in normal males. This embryo is typically intersexual.

2. Embryo no. 7–3 (fig. 27). Operated August 25, 1917. Piece of adult ovary grafted on a seven-day embryo. Opened September 4th. Age of embryo, 17 days; duration of graft, 10 days.

Result: Right gonad of the male type, 2.8 x 0.6 mm.; left gonad of the female type, 3.6 x 1 mm. Left gonad considerably larger than the right; its surface not smooth as in the normal testis, but rough like an ovary. Ducts poorly preserved. Parts of the müllerian ducts can be plainly recognized on both sides; whether the typical posterior enlargement was present cannot be determined.

3. Embryo no. 7–4. Conditions of the experiment same as in no. 7–3.

Result: Gonads of about the same length on both sides, 4.5 mm. Left gonad somewhat broader than the right and unlike a testis as to surface texture. Left müllerian duct of the female type; right duct shortened as in the normal female.

4. Embryo no. 2–5 (fig. 28). Operated June 21, 1917. Piece of ovary from a young hen grafted on a seven-day-old embryo. Opened, July 2nd. Age of embryo, 18 days; duration of graft, 11 days.

Result: Right gonad, 4.2 mm. long; left gonad, 5.4 mm. Peculiar black pigmentation over most of the surface of the left gonad, thicker over the anterior end. Left müllerian duct present; traces of middle and posterior portions of the right duct.

5. Embryo no. 17–1 (figs. 29, 30). Operated November 19, 1917. Piece of ovary from a month-old chick grafted on a thirteen-day-old embryo. Opened November 26th. Age of embryo, 20 days; duration of graft, 7 days.

Result: Gonads of the male type, but the right gonad very much smaller than the left one; right gonad, 2.6 mm. long; left gonad, 4.5 mm. long. Left gonad markedly pigmented, except over its posterior end. Wolffian ducts rather of the female type; only posterior portions of the müllerian ducts found.

6. Embryo no. 17–5 (fig. 31). Conditions of the experiment same as in preceding case.

Result: Very similar to no. 17–1, but size difference between the gonads not so great. Right gonad, 4.2 mm.; left gonad, 5.3 mm. Over half of the left gonad pigmented. Wolffian ducts of the male type. Left müllerian duct not well developed; slight trace of the right duct.

7. Embryo no. 29–7. Operated May 31, 1918. Piece of ovary from a ten-day chick implanted on an embryo of eleven days. Opened June 9th. Age of embryo, 20 days; duration of graft, 9 days.

Result: Gonads of the male type; right gonad, 4.5 mm. long; left, 5.5 mm. long. The size difference between the two gonads is greater than in the case of normal testes. Wolffian ducts of the male type. Traces of the degenerating müllerian ducts on both sides.

8. Embryo no. 18–9 (fig. 32). Operated November 23, 1917. Piece of ovary from a young hen grafted on an embryo of eight days. Opened December 4th. Age of embryo, 19 days; duration of graft, 11 days.

Result: Gonads of the male type in general; left one larger than the right; left gonad, 5 x 1.5 mm.; right gonad, 3.8 x 1 mm. Surface of the left gonad rough in texture, differing from that of the normal testis. Wolffian ducts of the male type; slight traces of the posterior portions of the müllerian ducts present on both sides.

9. Embryo no. 29–5 (fig. 35). Conditions of the experiment same as for no. 29–7. Age of embryo, 19 days; duration of graft, 8 days.

Result: Gonads of the female type, but the right gonad much elongated in form and larger than the normal right ovary; left gonad, 4.2 x 1.6 mm.; right gonad, 3 x 0.4 mm. Wolffian ducts more or less of the male type. Müllerian ducts developed on both sides; the posterior part of the right duct more developed than is the case in the normal female condition.

10. Embryo no. 1–7. Operated June 14, 1917. Piece of adult ovary grafted on an eight-day embryo. Opened June 24th. Age of embryo, 18 days; duration of graft, 10 days.

Result: Gonads of the female type; left gonad shortened and of irregular shape; right gonad elongated; left gonad 5; right gonad 3 mm. in length. Wolffian ducts rather of the male type; müllerian ducts developed to about the same degree on both sides.

11. Embryo no. 30–6 (figs. 33, 34). Operated June 5, 1918. Piece of ovary about 2 mm. square from a week-old chick grafted on a nine-day-old embryo. Opened June 14th. Age of embryo, 18 days; duration of graft, 9 days.

Result: Gonads of the female type, the right gonad remarkably slender; left gonad, 4.5 mm.; right gonad, 4 mm. in length. Wolffian and müllerian ducts of the female type.

12. Embryo no. 17–4 (fig. 36). Operated November 19, 1917. Whole ovary of a thirteen-day-old embryo grafted on a thirteen-day-old embryo. Opened November 26th. Age of embryo, 20 days; duration of graft, 7 days.

Result: Left gonad nearly like a normal ovary; right gonad does not resemble normal degenerating right ovary, but is more or less like a male gonad; left gonad, 6 x 2.3 mm.; right gonad, 2.5 x 1 mm. Wolffian and müllerian ducts of the female type.

13. Embryo no. 44–16. Operated April 5, 1919. Piece of ovary from young hen (five and one-half months old) grafted on a ten-day-old embryo. Opened April 15th. Age of embryo, 20 days; duration of graft, 10 days.

Result: Gonads of the female type, the right gonad larger than normal; left gonad, 5.5 x 1.6 mm.; right gonad, 3 mm. Wolffian and müllerian ducts of the female type, but the right müllerian duct better developed than in the normal female.

14. Embryo no. 30–9. Experimental conditions the same as in no. 30–6. Age of embryo, 19 days; duration of graft, 10 days. The graft grew very well; its size, 6.5 x 7.0 mm.

Result: Gonads of the female type in general, but smooth in texture similar to testes; shape also similar to that of testes; left gonad, 4.8 x 1.1 mm.; right gonad, 3.5 x 0.7 mm. Wolffian and müllerian ducts nearly as in the normal female.

d. Summary of intersexual characters produced by gonad grafts. The intersexual characters produced in chick embryos by the implantation of grafts of gonad tissue upon their chorio-allantoic membranes may now be summarized.

1. In affected embryos having gonads of the male type, the difference in length between the two gonads is greater than is the case with normal testes. The difference between right and left testes in normal embryos does not exceed 0.5 mm. (table 5). In several intersexual embryos, however, the difference in length of the two gonads exceeds 1 mm., and in extreme cases is still greater, as 1.8 mm. in embryo no. 10–5, 1.9 mm. in embryo no. 17–1, and 2.3 mm. in embryo no. 15–5. This length difference between the two gonads is abnormal for the male, but is characteristic of the female, where the left ovary is much larger than the right one. In this regard, then, the affected embryos may be considered as intermediate between normal males and females.

2. In several affected embryos, male-type gonads exist simultaneously with female-type müllerian ducts. Examples of this

condition are: embryos nos. 27–6, 31–3, 47–10, 43–3, among those grafted with testis, and nos. 27–4, 7–3, and 7–4, among those grafted with ovary. This condition represents a mixture of the characters of the two sexes.

3. It is important to note that the intersexual condition described under 2, where male-type gonads are combined with female-type müllerian ducts differs according to the kind of gonad grafted on the embryo in question. In the case of testis grafts, these intersexual embryos have gonads of the male type; that is to say, the two gonads are nearly of the same size. Examples: embryos nos. 27–6, 31–3, 47–10, 43–3, 27–5. On the other hand, such intersexual embryos when produced by ovary grafts have gonads which resemble ovaries; that is, the left gonad is considerably larger than the right one. Examples: embryos nos. 27–4, 7–3, 2–5. Not only are the gonads of the intersexual embryos different in size in such cases, but the shape and texture of the left ovary differs from that of the right, bearing a greater resemblance to the normal left ovary. The condition of the müllerian ducts in the intersexual embryos is also related to the kind of gonad which was responsible for the intersexual condition. Where the grafted gonad was a testis, the müllerian ducts of the affected embryos are undifferentiated (nos. 47–10, 43–3) and show signs of degeneration (as in nos. 27–6, 31–3, 27–5). Intersexual embryos produced by ovary grafts, on the other hand, retain the müllerian ducts, degenerative changes in the ducts are absent, and the left duct is better developed and more differentiated than the right one (nos. 27–4, 7–4, 2–5).

4. The right gonad of female-type embryos is larger than that of the normal female. The right ovary normally develops to a length of 1.5 to 2 mm., and then degenerates and disappears (table 4). On the other hand, in several of the affected embryos, the right gonad measures more than 3 mm., and in a few cases even more, as 3.3 mm. in embryo no. 17–7, 3.5 mm. in embryo no. 30–9, and 4 mm. in embryo no. 30–6. The right gonad in these embryos has a definite outline as compared with the fading outlines of the normal degenerating right ovary, and it resembles a rudimentary or degenerating testis.

5. The process of degeneration of the right müllerian duct is inhibited. In several cases the developed duct persists on the right as well as on the left side. Examples: embryos with testis grafts, nos. 31–3, 47–10, 43–3; embryos with ovary grafts, nos. 27–4, 7–3, 29–5, 1–7, 44–16.

6. The wolffian ducts typical of one sex are associated with the gonads or müllerian ducts typical of the opposite sex. This point is not, however, so striking as the other points.

7. A black pigmentation often appears on the male-type gonads. This is shown in embryos nos. 27–6, 43–3, 2–5, 17–1, and 17–5. It occurs with both testis and ovary grafts. Histological examination of such gonads reveals that the pigment granules occur not only on the surface, but extend into the interior of the organ and are mingled with the tissue of the stroma.

8. The histological changes in the affected gonads will be described in detail in a later paper, and hence will be but briefly summarized at this point. Histological examination shows that:

a. The male-type gonads are structurally modified testes. The tunica albuginea is abnormal and in some cases partially absent. Where present the connective tissue of which it is composed is much looser than in the normal albuginea. The stroma between the seminiferous tubules is also much less compact than in the normal testis. The seminiferous tubules are fewer in number and more irregular in arrangement than in normal testes. Within the tubules the germ cells are reduced in number and inactive or degenerating, as indicated by their staining behavior.

b. The female-type gonads are structurally modified ovaries. They are less modified from the normal than in the case of male-type gonads. In some cases a partial disappearance of the cortical layer was found; in others a degenerating or necrotic condition of the cords of second proliferation was noticeable.

c. The müllerian ducts which are associated with male-type gonads are very similar in structure to the developed ducts of normal females.

DISCUSSION

a. Action of sex hormones. The experiments presented in this paper show that the primary sexual characters of the chick embryo can be altered in several different ways through the implantation of testis and ovary tissue on the membranes of the embryo. Various degrees of intersexuality were produced. Whenever affected embryos resulted, it was always found that the grafts were growing (or had grown) and that a vascular connection had been established between the graft and the embryonic circulation. It is therefore not only a reasonable, but an unavoidable conclusion that the effect produced by the grafts on the embryo must have occurred by way of the circulation. It may indeed be conclusively stated that the grafts produced substances which were carried to the embryo in the blood stream and modified its sexual differentiation.

It may therefore be regarded as demonstrated that the gonads secrete substances which may be designated sex-hormones, which control or modify both primary and secondary sexual characters. The experimental work presented in this paper is in complete accord with Lillie's explanation of the free-martin condition in cattle, namely, that the free-martin is a female whose primary sexual characters have been modified in the male direction by the action of hormones originating in the testes of the male cotwin. My work shows that testicular and ovarian secretions actually do have such modifying effects on the primary sexual characters.

b. Specific function. It is necessary to determine whether or not the modifications observed in these experiments are a specific reaction to gonad secretions or whether they might not be produced in response to secretions from other organs. This point has been subjected to extensive experimental tests with the following results.

Pieces of organs other than gonads were grafted on chick embryos in the same manner as in the case of gonads. The organs used were: thymus, thyroid, spleen, liver, kidney, and some others. Experiments of a similar kind have also been performed in this laboratory by Mr. B. H. Willier, and I had the privilege of examining Mr. Willier's material; I am indebted to him for his kindness in this matter. From my own material and that of Mr. Willier there were available the following embryos: twelve embryos from thymus grafts; eight embryos from thyroid grafts; two embryos from thyroid and thymus grafts, i.e., pieces of both organs implanted on the same embryo; nine embryos from spleen grafts; three embryos from kidney grafts, and two from liver grafts. It is understood that in all of these cases the grafts grew.

In spite of the fact that grafts from these organs grow more readily and faster and attain a larger size than grafts from gonads, in no case was any effect produced upon the urinogenital systems of the embryos which had borne the grafts.[2] All of the embryos thus obtained are normal males or females, as regards their reproductive systems. I do not wish to imply that these grafts may not have produced some effects upon the embryos, but it may be affirmed with certainty that they had no effect upon the sexual differentiation.

In view of these facts, it may be conclusively stated that the action of the secretions of the gonads upon the primary sexual characters is specific. Only gonad secretions are capable of modifying the sexual characters in experiments of this kind.

c. *Effect of duration of grafts.* An examination of the relation between the duration of the graft and the extent of modification of the embryos will give us some idea of the length of time during which the sex-hormones must act in order to produce an effect. The duration of the graft, that is, the length of time which has elapsed between operation and examination, is a rough measure of hormone dosage employed in each case; the longer the duration, the greater the dosage, presumably. Other factors must, however, be considered. Chief among these is the degree of growth of the graft. Although the same procedure was followed in each operation, and approximately the same amount of tissue implanted, the mode and degree of growth of the grafts was very variable. One graft would grow readily; while another would become partly necrotic. Another factor to be considered is the condition of the embryo on which the graft is implanted. Even if the grafts grow equally well and remain upon the embryos an equal length of time, they will not necessarily produce the same degree of effect, since the embryos themselves vary as to age, and presumably also in general metabolic condition, degree of resistance to foreign tissues, and so on. In consequence of these variable factors, it is difficult to determine in experiments of this kind the relation between amount of dosage and degree of modification of the embryos. In general, however, it may be said that grafts must remain upon the embryo for a certain minimum length of time in order to bring about a modification of its sexual characters. This minimum length of time is about one week.

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[2] In two cases, however, one with spleen graft and one with liver graft, the left testis showed the same black pigmentation as in the case of several gonad grafts.

d. Effect of age of birds furnishing the grafted gonads. Another point of interest which arises in connection with these experiments is whether the age of the individuals from which the gonads to be grafted were taken bears any relation to the degree of modification produced. The age of the birds whose gonads were removed for grafting varied in these experiments from embryos eight or nine days in age up to adult fowls. It has seemed to me, however, that in many cases, gonads obtained from young chicks, between the ages of one week and one month, were more effective in producing modifications in the embryos on which they were grafted than gonads from birds of other ages.

e. Effect of the age of the embryo at the time of grafting. The experiments yielded definite results upon this point. As has already been stated in the section dealing with the growth of the grafts, the best results were obtained when the grafts were implanted on embryos during the second week of incubation; that is, from the seventh to the thirteenth day. The reason for this fact is not difficult to find. In the chick embryo the differentiation of the sexes appears on about the sixth or seventh day of incubation and progresses rapidly to the eleventh and twelfth days. This period, approximately the second week of incubation, is then the period of active sexual differentiation. It is characterized by the differentiation of the seminiferous tubules in the male and the cortical cords in the female, by the retrogression of the müllerian ducts in the male and the degeneration of the right ovary and duct, and differentiation of the left duct into an oviduct in the female.

As a consequence of the developmental time relations with reference to sexual differentiation, it is reasonable to expect that the grafts will produce the greatest effect during the second week of incubation. This period is the period of sexual differentiation; the sexual characters have not yet become fixed, and hence it is reasonable to suppose that they may be controlled by introduced factors. It is probable that the grafts would be still more effective if they could be implanted before sexual differentiation begins, that is, on the fourth or fifth day; but as already stated, grafts do not grow on such early embryos, owing to the fact that the allantoic circulation has not yet been established.

f. Degree of reversibility. Examples of natural or experimental reversals of sex have already been quoted. Such cases are those of the free-martin (Lillie), the guinea-pig and rat (Steinach, Moore), Bonellia (Baltzer), and Crepidula (Gould). Intersexes or sex intergrades have been obtained in pigeons by Riddle, in the gypsy-moth by Goldschmidt, in Simocephalus by Banta ('16 a, b), and in Drosophila by Sturtevant ('20). In these cases various degrees of reversal of sex were noted, and this reversal was correlated in a quantitative way with the degree of development of the sex organs. The cases of Lillie, Steinach, and Moore are the only cases in which the sex reversal was explained as the direct result of the action of sex-hormones produced by the gonads.

The present experiments bear upon the problem of the reversibility of sex. Intersexual conditions were produced in chick embryos by grafting pieces of testis and ovary upon their membranes. The interpretation of the intersexual individuals is rendered very difficult in the case of experiments of this kind, since we do not with certainty know the sex of the embryos on which the grafts were implanted. In other words, we do not know what sex the embryo would have been if allowed to develop without experimental interference. Nevertheless, examination of these embryos permits us to draw with reasonable certainty some conclusions concerning their original sex and the degree to which this sex had been modified in the direction of the opposite sex.

It is highly probable that certain of these embryos which received testis grafts were originally females and were subsequently modified in the male direction through the presence of the testis graft. This appears to be the case in embryos nos. 27–6, 31–3, 47–10, 43–3, 27–5. Certain structural features of these embryos indicate that they had originally developed and differentiated to a certain extent in the direction of femaleness. This is evidenced principally by the presence and degree of differentiation of the müllerian ducts; both conditions are similar to those of the female. It is difficult—in fact, impossible—to account for the condition of the müllerian ducts in these embryos except on the basis that the embryos were originally females and had differentiated to this extent in the female direction. There is no possibility that the presence of testis grafts on such embryos could have induced the differentiation of the müllerian ducts. Hence we are compelled to believe that these embryos were originally females. But they possess gonads of the male type. There is no escape from the conclusion that their gonads, originally ovaries, have been greatly modified in the male direction through the action of the secretion from the testes grafted on their membranes.

Certain other embryos of the female type show modification in the male direction, as embryos nos. 17–7, 22–13, 22–9. In these cases the embryos were eleven and thirteen days old when the testis graft was implanted on them. They had therefore attained considerable differentiation in the female direction before the testis hormone acted upon them. In these cases the right ovary persisted—a result which must be ascribed to the action of the testis hormone. We may say that the testis secretion cannot cause the disappearance of the right ovary, or, to put the matter in another way, it tends to make both gonads develop equally.

In the case of ovary grafts, certain embryos were obtained which were probably originally males and which have been modified in the female direction. Such cases are nos. 27–4 and 2–5. These embryos have gonads of the male type, but the left gonad is considerably larger than the right one. We may conclude that these gonads began to develop in the male direction, but after coming under the influence of the ovary graft developed in the female direction. The müllerian ducts of these embryos also show a modification in the female direction; the left duct is better developed and more differentiated than the right one. The left duct does not, moreover, show any signs of degeneration. These conditions can again be interpreted as the consequence of the action of an ovarian secretion upon an originally male embryo. Similar conditions are exhibited by embryos nos. 17–1, 17–5, and 18–9, which also received ovary grafts. These embryos have gonads of the male type, but the left one is again larger than the right one and there are no müllerian ducts. These embryos may be regarded as originally male embryos which were less markedly modified in the female direction than is the case with the two embryos just discussed, nos. 27–4, and 2–5. The reason for this becomes apparent when the experimental conditions in these cases are considered. Grafts were made on embryos nos. 17–1, 17–5, and 18–9 when they were eleven and thirteen days old. Hence they had already undergone considerable differentiation in the male direction, and it is not to be expected that the ovarian secretion could produce such marked effects at that late stage of development as in the cases of nos. 27–4 and 2–5, where the grafts were implanted at nine and seven days, respectively.

Female-type embryos nos. 30–6, 29–5, 1–7, 17–4, 30–9, and 44–16, on which ovary had been grafted, are somewhat puzzling. These embryos have a persistent right gonad. It is difficult to say whether these embryos were originally male and have been markedly transformed in the female direction or whether they were originally females in which the gonads have been stimulated to supernormal development by an excess of ovarian secretion.

In several male-type embryos—nos. 27–6, 43–3, 2–5, 17–1, 17–5, and 44–2—a black pigmentation was present in the gonads. It is a curious and interesting fact that in every case except no. 27–6, the black pigmentation is on the left gonad, and in every case it is on the anterior portion of the gonad. In number 27–6 the right gonad is also pigmented, but to a much less degree than the left one. I am unable to suggest any explanation of the significance of the pigmentation. Normal testes and ovaries are, of course, never pigmented. Bond ('14) and Pezard ('18) described the occurrence of pigmentation in the ovary of the pheasant in correlation with degenerating processes. In two cases of non-gonad grafts, one case with spleen and the other with liver, I found an exactly similar pigmentation of the left testis. It would therefore appear that this occurrence is not a specific effect of the sex hormones.

Although I have not a large number of cases and although the analysis of the results must in the nature of the experiments be largely of an inferential kind, I believe that these results furnish strong evidence that the sexual characters are reversible and that these characters after having differentiated to a certain extent in the direction of one sex may be altered and modified in the direction of the opposite sex.

g. Conclusion. Consideration of the experimental data leads us to the following conclusion. The testis and ovary of the chick secrete certain physiological substances, which we may designate sex-hormones. When these secretions are introduced into the body of an embryo they exercise a specific effect upon its reproductive system. The development and differentiation of one sex is stimulated by the secretion of the gonad of the same sex and inhibited by the secretion of the gonad of the opposite sex. By means of these secretions, the differentiation of sex in the chick can be controlled to some extent. It may furthermore be stated that the two sexes bear a quantitative and not a qualitative relation to each other.

SUMMARY

1. The purpose of this investigation was to determine whether or not sexual differentiation is controllable and reversible through the action of sex-hormones.

2. The common fowl was used as material.

3. A small piece of testis or ovary was grafted on the chorio-allantoic membrane of a developing chick. Testes and ovaries used for grafting were taken from birds varying in age from an eight-day-old embryo to the adult. The embryos upon which the grafts were implanted varied in age from two to sixteen days at the time of the operation.

4. In a number of cases the grafts grew and established vascular connections with the allantoic blood-vessels.

5. A certain number of the embryos upon which the gonad grafts had proved successful exhibited a greater or less degree of modification of their reproductive systems. The most important alterations were these:

a. The simultaneous existence of gonads of the male type and differentiated müllerian ducts of the female type.

b. An alteration of the normal size ratio of the two testes. Whereas the two testes in the normal male are of the same size at hatching, in many of these cases the left testis was markedly larger than the right one.

c. Persistence of the right gonad in female-type embryos. The right gonad disappears in normal female embryos.

6. In normal chicks it was found that the wolffian ducts are persistent in the female.

7. Grafts of liver, spleen, thyroid, thymus, and other organs on chick embryos produce absolutely no modifications of the reproductive system.

8. The results demonstrate that the testis and ovary produce secretions which have definite and specific physiological functions and which are capable of modifying the primary sexual characters.

9. It is shown by these experiments that sexual differentiation may be reversed in the chick. This result supports the explanation advanced by Lillie to account for the production of the free-martin in cattle.

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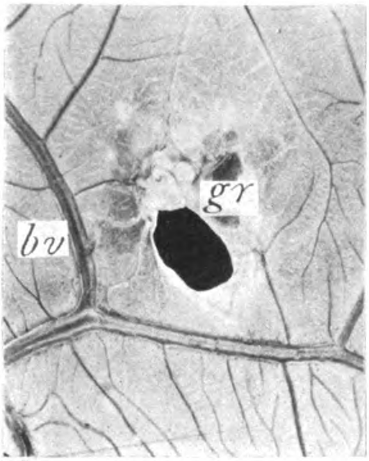
PLATE 1

EXPLANATION OF FIGURES

1 Example of a graft growing on the chorio-allantoic membrane, showing also the vascular connections. Ovary graft; embryo no. 17–5. *Gr*, graft; *bv*, blood-vessel. × 3.

2 Section of the same graft. *Fo*, follicles. Oc. 2 × obj. 1/12.

3 Section of normal adult ovary. *Fo*, follicles. Oc. 2 × obj. 1/12.



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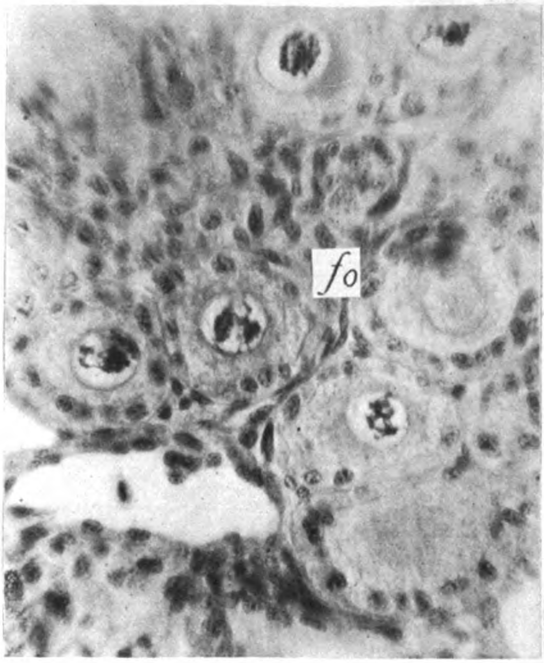

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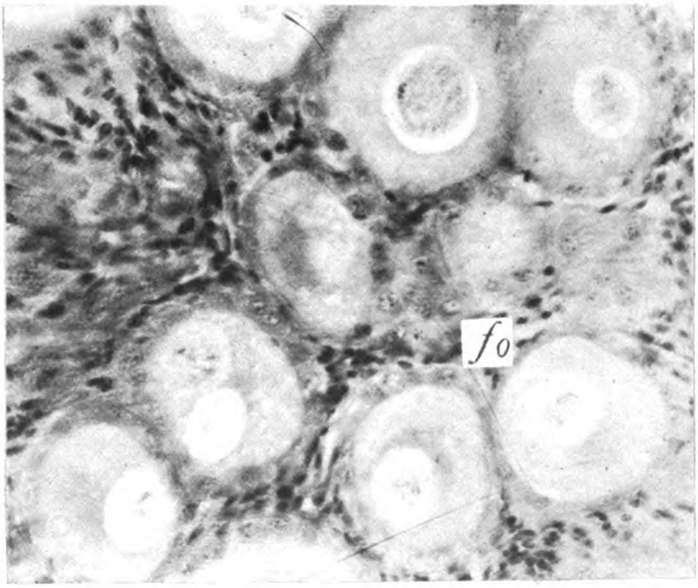

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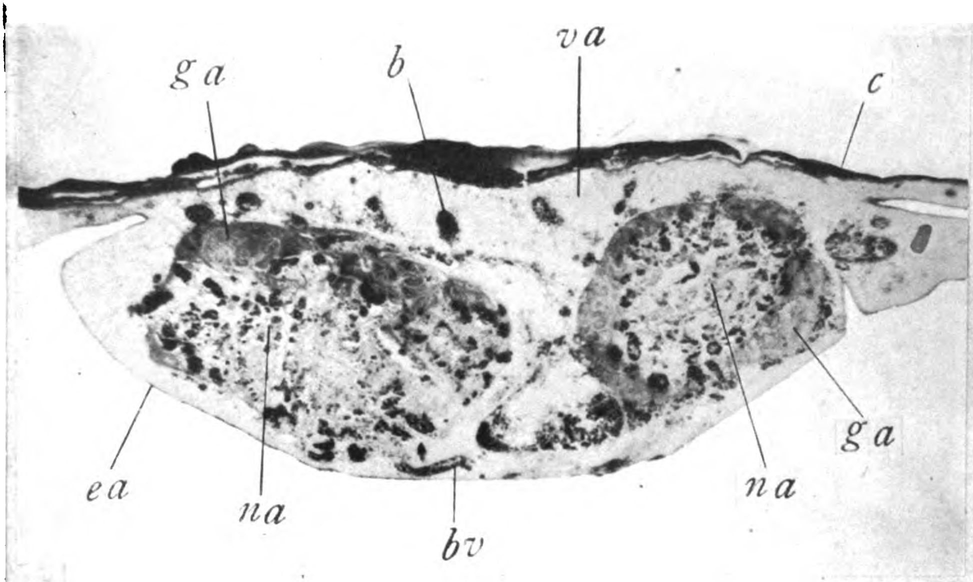
PLATE 2

EXPLANATION OF FIGURES

4 Example of a growing graft. Testis graft; embryo no. 17-7. *Gr*, graft; *bv*, blood vessel. × 4.

5 Section of a testis graft. Embryo no. 20–1. *Ec*, ectoderm of chorion; *ea*, entoderm of allantois; *bv*, blood-vessel; *va*, vascular area; *ga*, growing area; *na*, necrotic area. Oc. 4 × obj. 32.

6 Portion of a growing area of the same section, magnified. *St*, seminiferous tubules; *mi*, mitotic figures. Oc. 2 × obj. 1/12.



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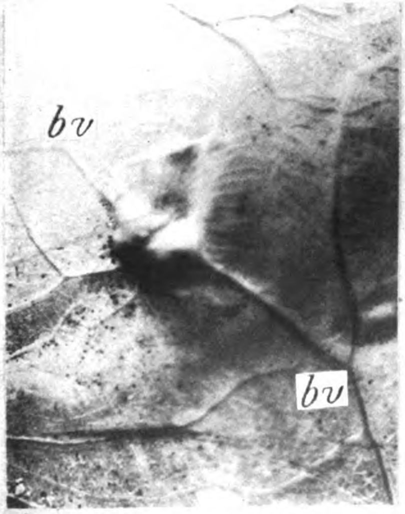

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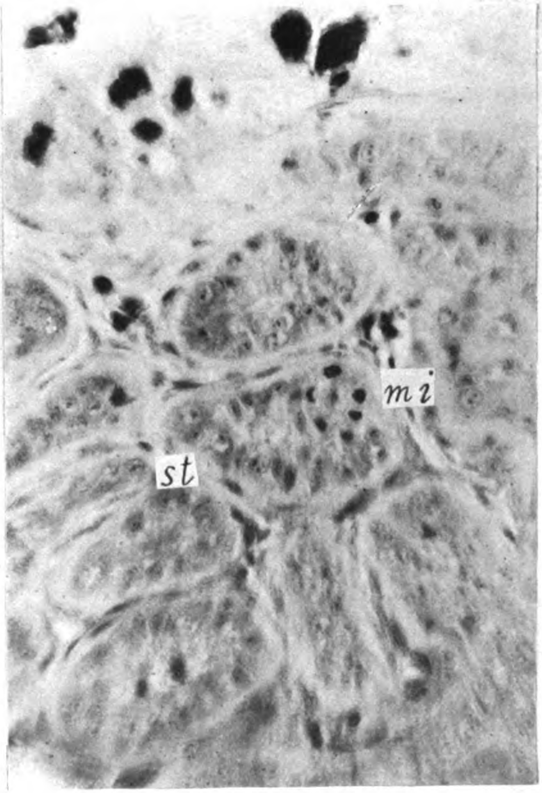

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PLATE 3

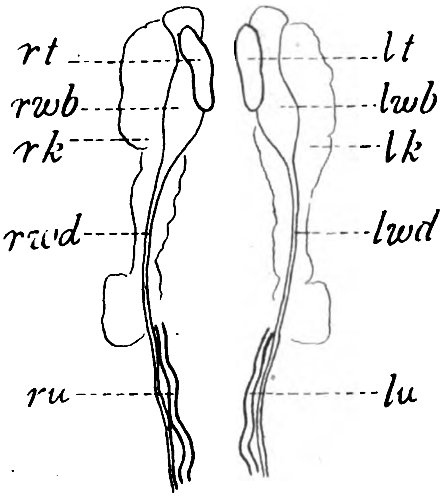
EXPLANATION OF FIGURES

7 Urinogenital system of a normal male embryo, eighteen days old. *Rt*, *lt*, right and left testis; *rwb*, *lwb*, right and left wolffian bodies; *rwd*, *lwd*, right and left wolffian ducts; *ru*, *lu*, right and left ureters; *rk*, *lk*, right and left kidney. × 4.

8 Diagram of the urinogenital system of the same embryo.

9 Urinogenital systems of a normal female embryo, eighteen days old. *Lo*, left ovary; *rmd*, *lmd*, right and left müllerian ducts; other abbreviations as in figure 7. × 4.

10 Diagram of the same embryo.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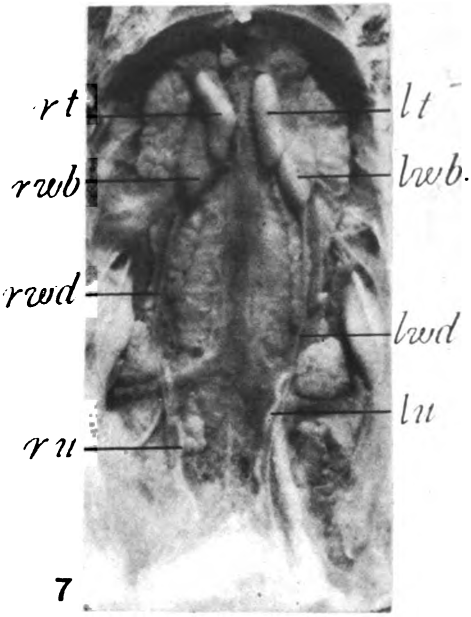
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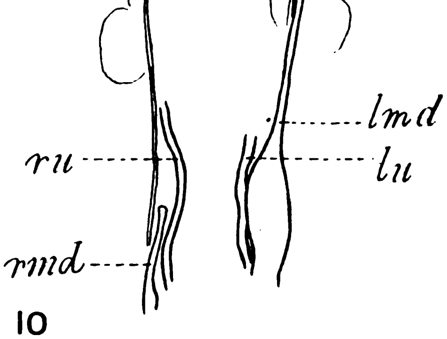
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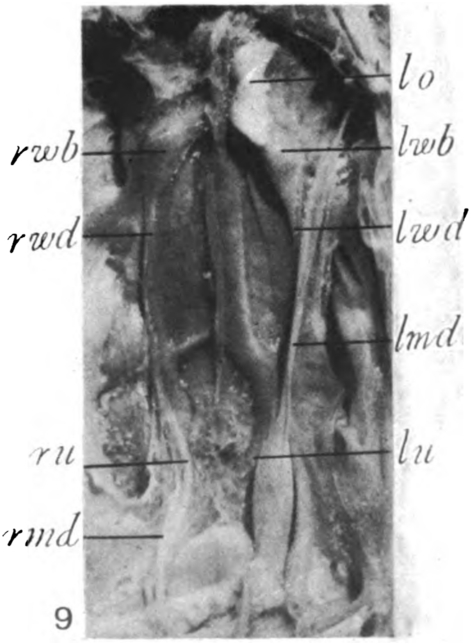

lo
rwb
lwb
rwd
lwd
lmd
ru
lu
rmd

9

PLATE 4

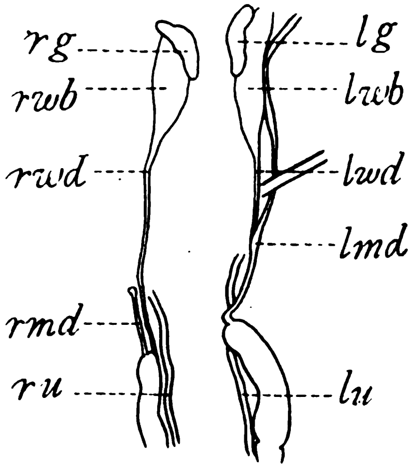
EXPLANATION OF FIGURES

11 Urinogenital system of embryo no. 27–6, modified by testis graft. *Rg*, *lg*, right and left gonads; *rwb*, *lwb*, right and left wolffian bodies; *rwd*, *lwd*, right and left wolffian ducts; *rmd*, *lmd*, right and left müllerian ducts; *ru*, *lu*, right and left ureters. Same abbreviations used in all subsequent figures. × 3.

12 Diagram of the urinogenital system of the same embryo.

13 Urinogenital system of embryo no. 31–3, modified by testis graft. × 3.

14 Diagram of the urinogenital system of the same embryo.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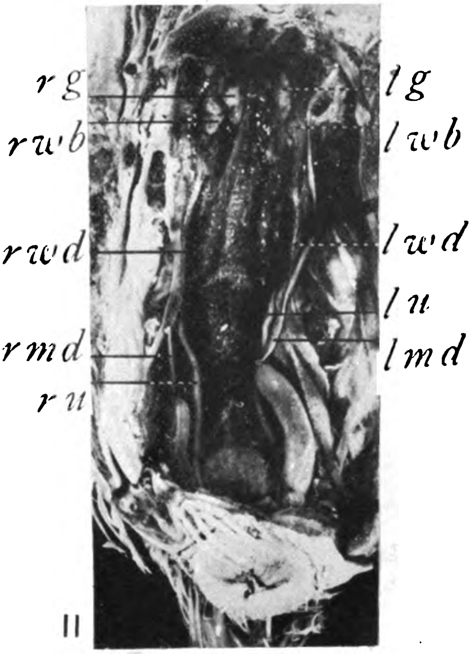
12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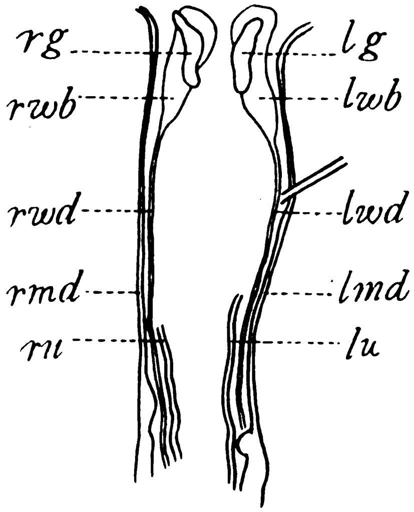
11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lg
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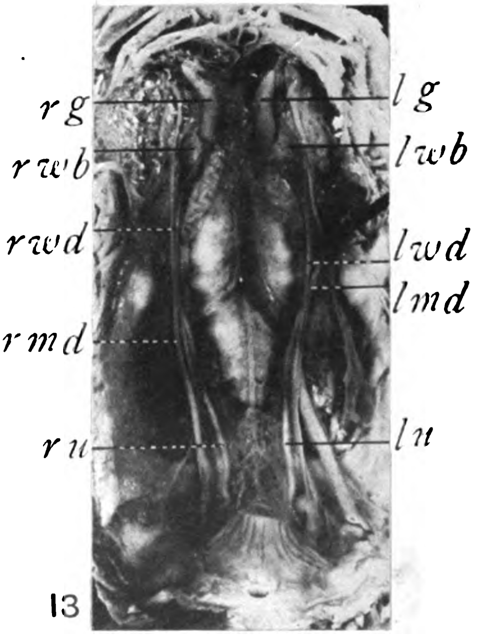
lu

14


rg
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ru
lg
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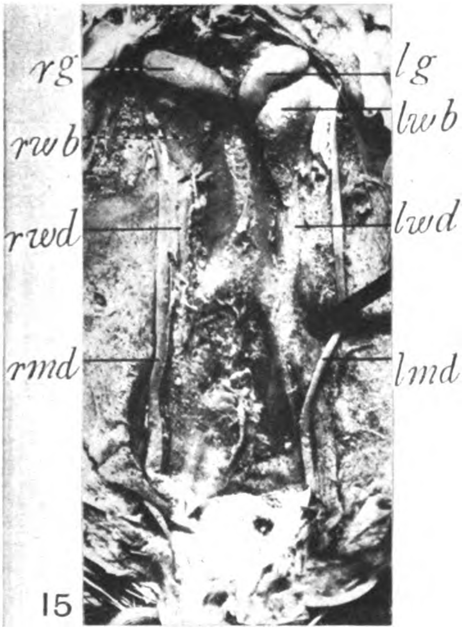
lmd
lu

13

PLATE 5

EXPLANATION OF FIGURES

15 Embryo no. 47–10, modified by testis graft. × 3.
16 Embryo no. 43–3, modified by testis graft. × 4.
17 Embryo no. 27–5, modified by testis graft. × 3.
18 Embryo no. 15–5, modified by testis graft. × 3.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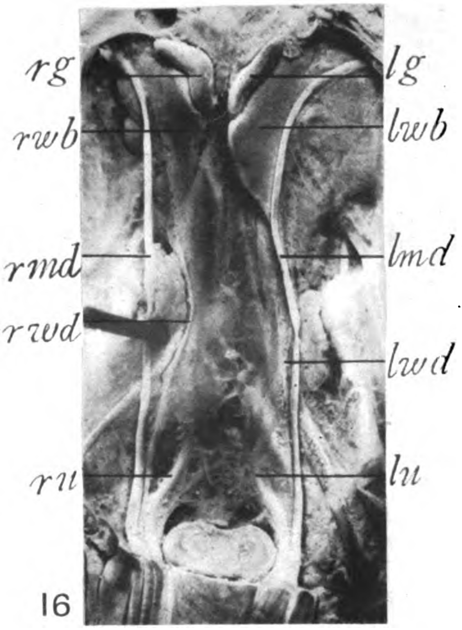
15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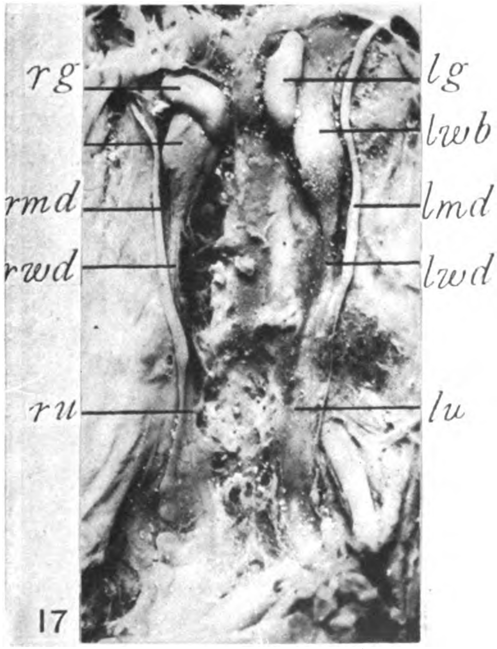
lu

16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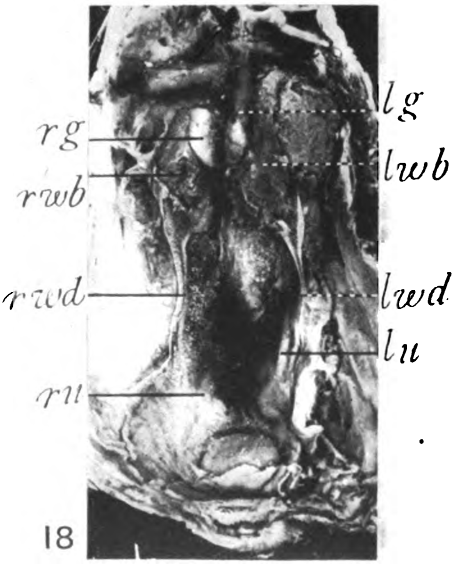
lg
rg
lwb
rwb
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lwd
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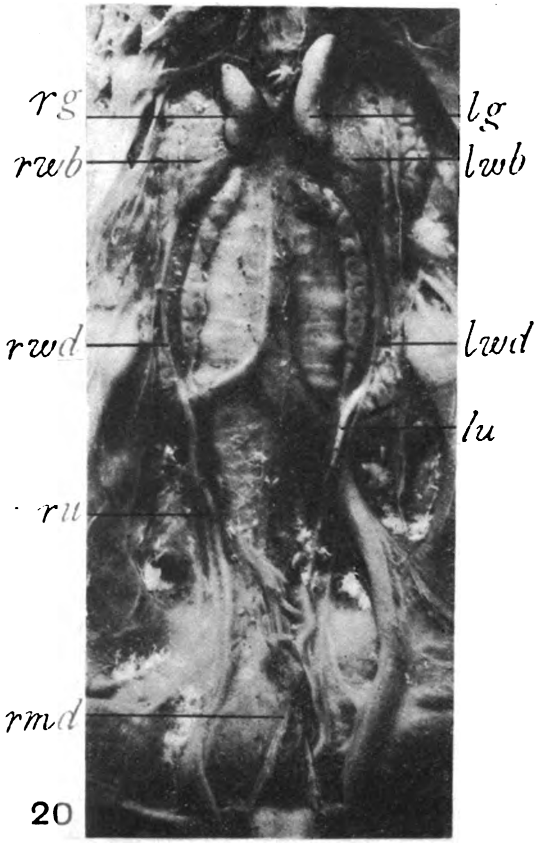
18

PLATE 6

EXPLANATION OF FIGURES

19 Embryo no. 45-6, modified by testis graft. × 4.
20 Embryo no. 45-18, modified by testis graft. × 4



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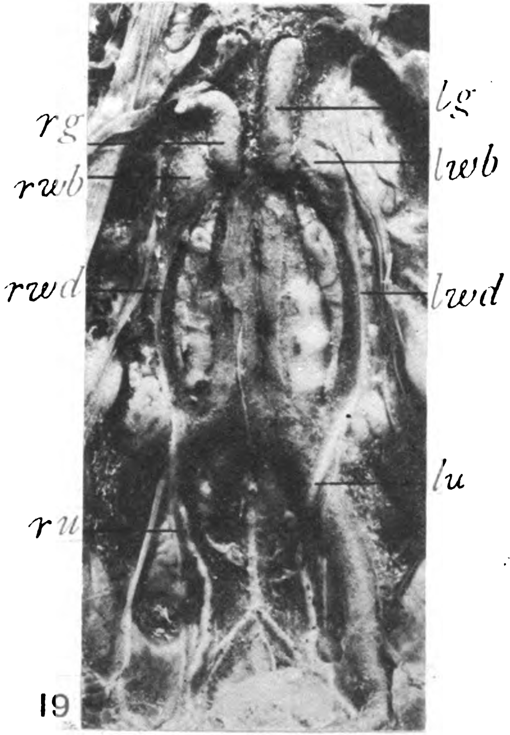
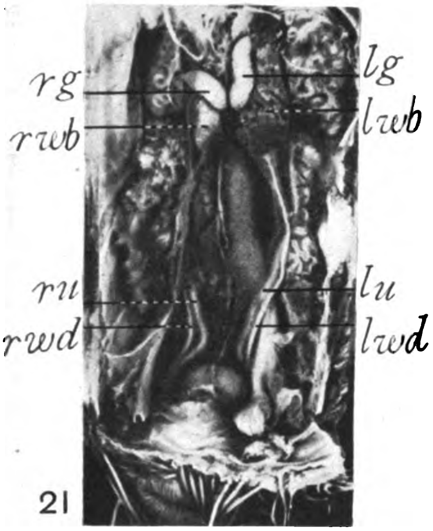


19

PLATE 7

EXPLANATION OF FIGURES

21 Embryo no. 8-5, modified by testis graft. × 3.
22 Embryo no. 22-13, modified by testis graft. × 4.
23 Embryo no. 17-7, modified by testis graft. × 4.
24 Diagram of the urinogenital system of the same embryo.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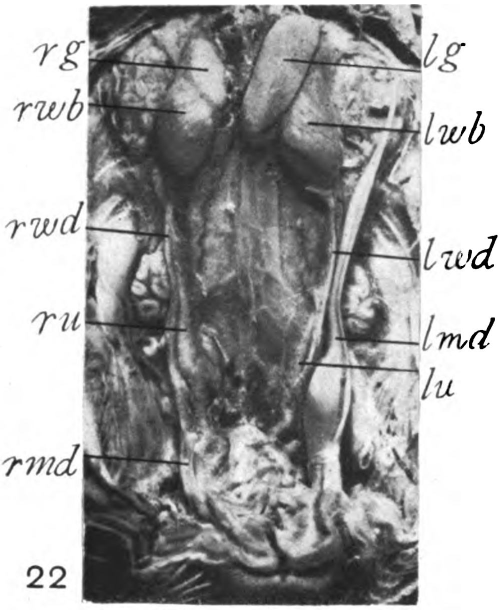
21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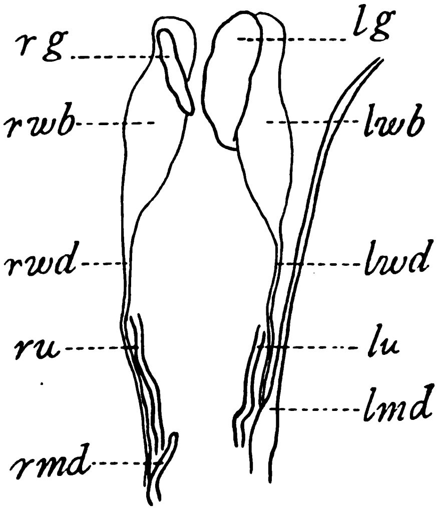
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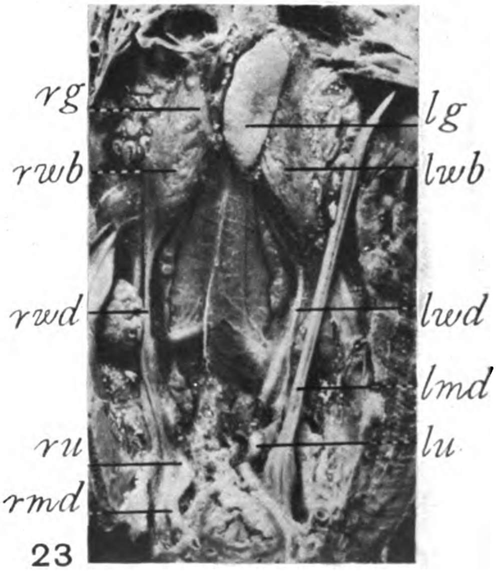
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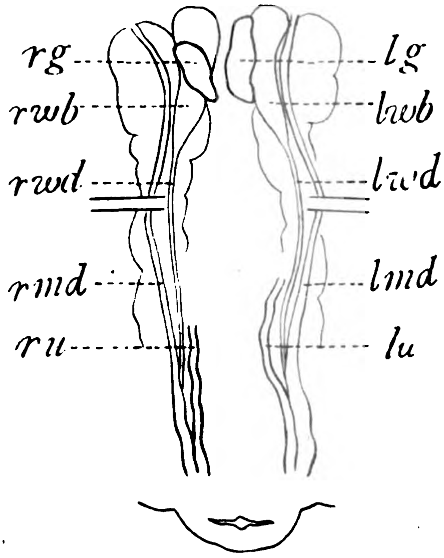
23

PLATE 8

EXPLANATION OF FIGURES

25 Embryo no. 27-4, modified by ovary graft. × 3.
26 Diagram of the urinogenital system of the same embryo.
27 Embryo no. 7-3, modified by ovary graft. × 3½.
28 Embryo no. 2-5, modified by ovary graft. × 4.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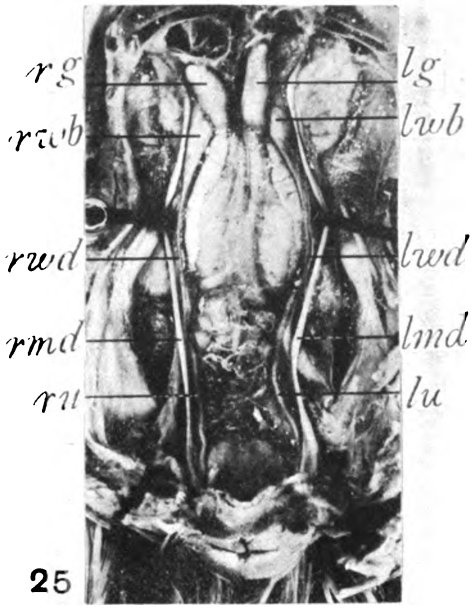
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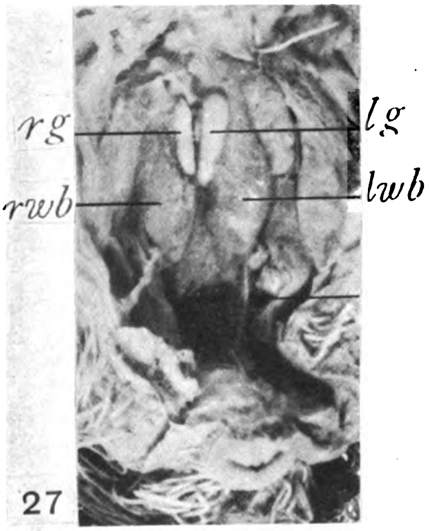
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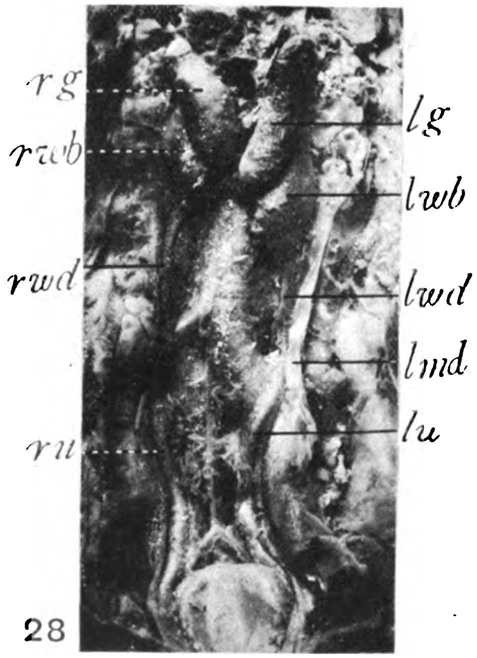
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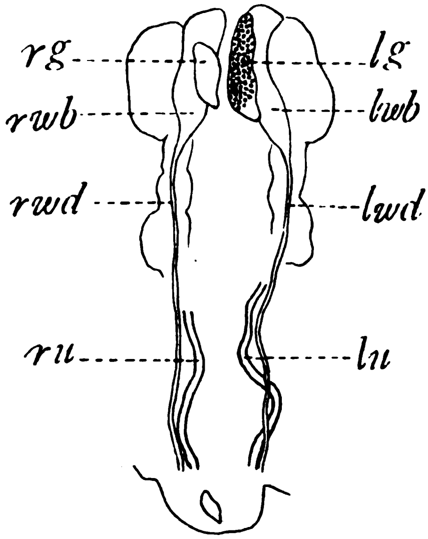
PLATE 9

EXPLANATION OF FIGURES

29 Embryo no. 17–1, modified by ovary graft. × 3.
30 Diagram of the urinogenital system of the same embryo.
31 Embryo no. 17–5, modified by ovary graft. × 3.
32 Embryo no. 18–9, modified by ovary graft. × 3.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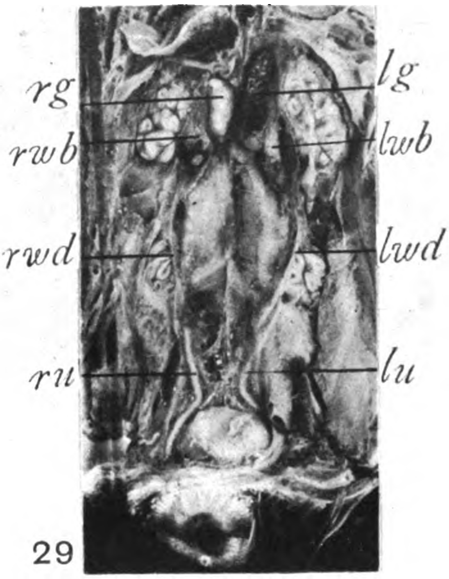
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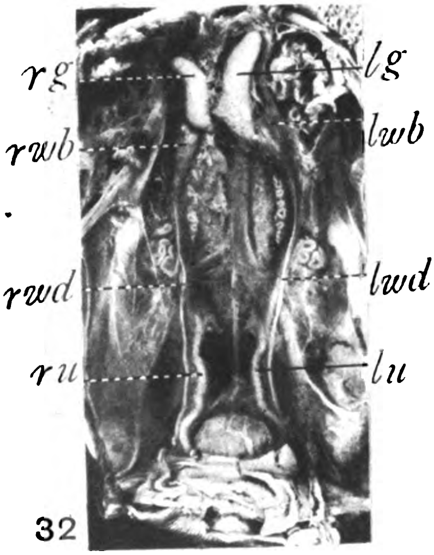
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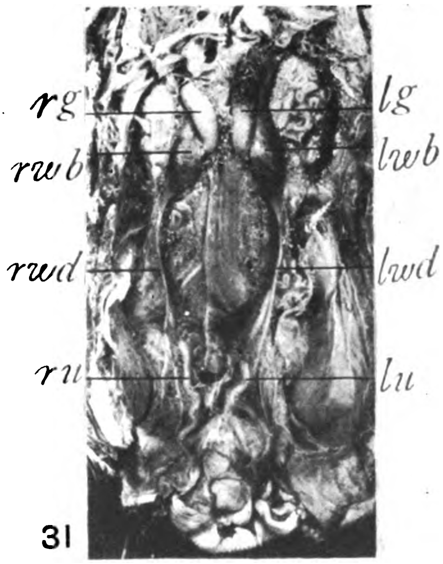
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31

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